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INFLUENCE OF TEMPERATURE ON EFFECTIVENESS OF LEAD ARSENATE AGAINST LARVAE OF THE JAPANESE BEETLE IN THE SOIL

By Walter E. Fleming, Entomologist, and Warren W. Maines,
Scientific Aide, Division of Fruit Insect Investigations

INTRODUCTION

Since 1922, when it was discovered that the introduction of lead arsenate into soil would kill larvae of the Japanese beetle, *Popillia japonica* Newman (3, 4),[1] applications of this material have been used successfully in controlling larvae of this and allied species. The larvae ingest the arsenical as they burrow through the poisoned soil or while feeding on rootlets growing in the soil.

The insecticidal action of lead arsenate in the soil is complex, being modified by interacting physical, chemical, and biological factors. The physical condition of the soil, the presence or absence of various constituents in the soil, the temperature, the moisture, and the type of vegetation have an effect on the distribution and the fixation of the chemical and on the activity of the insect. To be effective, the poison must be distributed in an active form in the layer of soil in which the larvae are burrowing or feeding. Any factor which modifies the availability or distribution of the poison or changes the activity or depth of the larvae in the soil has a bearing on the success of the treatment.

It has been recognized for several years that the temperature of the soil was an important factor, but until recently the relation between temperature and insecticidal action had not been definitely established. Experiments conducted since 1941 have established this relationship with the newly hatched and third-instar larvae.

1/ Underscored figures in parentheses refer to Literature Cited, p.11.

EFFECTIVENESS OF LEAD ARSENATE AT DIFFERENT TEMPERATURES

In their natural habitat in the field the larvae are subjected to daily and seasonal variations in the temperature of the soil. Under such conditions it is difficult to establish definitely the exact relationship between the temperature and the poisoning. Experiments on the effect of temperature were therefore conducted in the laboratory as well as in the field.

In the laboratory experiments at constant temperatures, the lead arsenate was thoroughly mixed with sifted sassafras sandy loam at the rates of 20.8 and 41.6 grams per cubic foot, which was equivalent to incorporating, respectively, 500 and 1,000 pounds of the chemical with the upper 3 inches of an acre of soil. The soil and the chemical were mixed by passing them several times through a gyratory riddle. The treated soil was placed in trays and brought to optimum moisture, and grass seed was sown. The trays used for newly hatched larvae were 12 inches square and 2 inches deep; those used for third instars were 18 inches square and 3-3/4 inches deep. Each experimental unit consisted of two or more trays of unpoisoned soil and two or more trays of each arsenical treatment.

Experimental units were placed in chambers and maintained, with a variation of $\pm$ 1 degree, at 50°, 60°, 70°, and 80° F. during the course of the investigation. A few days after an experimental unit had been put into the chamber, 200 newly hatched larvae or 200 to 400 field-collected third instars were introduced into each tray of untreated and treated soil. The newly hatched larvae were 2 days old when introduced into the soil; the age of the third instars at the beginning of the experiments was unknown, but it was evident that there was some variation in the degree of development of the individuals. Experiments certainly could have been conducted more precisely with individuals of a known age, but it was not possible to obtain a sufficient number of third instars of known age for this work.

At intervals which varied with the temperature, the larvae were removed from the soil and a record was made of the number of dead and living individuals in each tray. The living larvae were then returned to the soil and each tray was reseeded and watered. The experiments were repeated several times with different batches of larvae, a total of 4,800 newly hatched larvae and 7,800 third instars being used in this phase of the investigation.

The mortality of larvae in soil containing lead arsenate is the result of poisoning and, to some extent, other factors such as bacterial disease, nematodes, and injury. As the mortality in the untreated soil rarely exceeded 20 percent even in experiments of long duration, it was evident that death from causes other than poisoning was not an important factor in this investigation. The percentage of larvae poisoned by each treatment was determined by the formula

$$\text{Percent poisoned} = \frac{\text{Number alive in untreated soil} - \text{Number alive in treated soil}}{\text{Number alive in untreated soil}} \times 100$$

Scatter diagrams showing the relationship between the mortality of newly hatched and third-instar larvae and the number of days the insects had been in the treated soil were prepared for the 500- and 1,000-pound treatments at each temperature. Then, following the method outlined by Ezekiel (1), free-hand curves (figs. 1 and 2) were fitted to the average points.

The difference between each experimentally determined mortality and the mortality estimated from the average curve was determined. From these deviations the average standard deviation was found to be 3.3 percent and 3.7 percent for the 500- and the 1,000-pound treatments, respectively, against the newly hatched larvae and 13.9 and 12.5 percent, respectively, for these treatments against the third instars. The individual experimental results were not entered on these graphs because of their large number. The average standard deviations, indicated as standard errors on the graphs, will give the reader a clue as to how these values were scattered about the average curves.

It was evident that the effectiveness of the arsenical treatments against both ages of larvae was modified profoundly by the temperature. With the newly hatched larvae the 500-pound treatment required about 13 days at 50° F. to kill half of the larvae, 8 days at 60° F., and 6 days at 70° F. The 1,000-pound treatment accomplished this in 11 days at 50° F., 6 days at 60° F., and 4 days at 70° F. The third instars were considerably more resistant to poisoning. To kill half the third instars with the 500-pound treatment required more than 160 days at 50° F., 57 days at 60° F., 35 days at 70° F., and 25 days at 80° F.; with the 1,000-pound treatment, 45 days were required at 50° F., 23 days at 60° F., 15 days at 70° F., and 11 days at 80° F.

RELATIVE VELOCITY OF POISONING AT DIFFERENT TEMPERATURES

There seemed to be a definite relationship between the temperature and the velocity of poisoning with a given treatment of lead arsenate. As the reciprocals of the time required to obtain a definite level of mortality are a convenient measure of the relative velocity of poisoning, the data were converted to this form for further study.

From the average curves given in figures 1 and 2, the number of days required to poison any required percentage of the larvae could be determined. Then the reciprocals of values in multiples of 10 were obtained, and for each level of poisoning the reciprocals were plotted against the corresponding temperatures. A straight-line relationship seemed to exist between the relative velocity of poisoning and the temperature. The slopes of these lines were determined by the method of least squares. It was found that the curves for the newly hatched larvae intersected the X axis at points ranging from 38° to 42° F. and the curves for the third instars between 35° and 47°. The average point of intersection in both cases was approximately 40°. As these variations may be attributed to errors in determining the slopes of these lines, it was decided to modify the slopes slightly so that they would intersect the axis at 40°. With this modification, the relative velocity of poisoning of the larvae is presented somewhat diagramatically in figures 3 and 4.

The velocity of poisoning of both the first and third instars increased progressively with the increment in the temperature. On the average, at 60° F. the velocity of poisoning was double that at 50°, at 70° it was tripled, and at 80° it was quadrupled. The rate of poisoning of the newly hatched first instars was about four times that of the fully grown third instars.

THRESHOLD TEMPERATURE OF POISONING

The minimum temperature at which larvae are sufficiently active to ingest lead arsenate is an important factor in that it limits the period of insecticidal action in the fall and in the spring. The threshold temperature above which the larvae begin to ingest perceptible amounts of food has not been determined experimentally. Ludwig (5) considered 50° F. as below the threshold of development of the larvae, and Fox (2) suggested that larvae were inactive below this temperature. However, since it was possible to poison an appreciable number of newly hatched and third-instar larvae at a constant temperature of 50° F., it was evident that the threshold of feeding and possibly of movement is below this temperature.

It may not be possible to determine experimentally the temperature above which poisoning will be perceptible, but it is possible from the reciprocal curves given in figures 3 and 4 to obtain an empirical estimate of this temperature. The fact that the curves intersected the X axis at approximately 40° F. suggests that this is about the temperature above which larvae are poisoned by lead arsenate in the soil. This temperature has been accepted tentatively as the threshold of poisoning of larvae of the Japanese beetle by lead arsenate.

POISONING AND THERMAL SUMMATION

With the close relationship prevailing between the mortality and temperature, it seemed that the same relationship should exist between the mortality and the summation of the day-degree units above the threshold of poisoning. With 40° F. as the threshold, it was found with the 500- and the 1,000-pound treatments at constant temperatures that the products of the number of days and the number of degrees above the threshold were practically constant values for each level of poisoning. It was evident that the poisoning was closely correlated with the summation of the day-degree units above 40°.

The mortalities of newly hatched and third-instar larvae obtained with the 500- and the 1,000-pound treatments at constant temperatures were plotted against the summations of the day-degree units above the threshold. The scatter diagrams showing this relationship are presented in figures 5 and 6. The average curves and their standard deviations were derived in the same manner as those shown in figures 1 and 2. In this case the average standard deviation is expressed as broken lines above and below each curve. It was found that within the range of normal experimental error these curves were an adequate expression of this relationship.

It was not possible to conduct experiments with newly hatched larvae in the field, but such experiments were conducted with third instars. In October 1941 a group of 270 trays of established turf growing on unpoisoned soil and on soil containing 500 and 1,000 pounds of lead arsenate per acre were infested with 5,400 third instars. These trays, which were 18 inches square and about 4 inches deep, were exposed on the ground to the weather during the course of the experiment. A record was made daily of the temperature of the soil at a depth of 1 inch. At intervals of 2, 4, 8, 12, 16, 20, 24, 28, and 30 weeks following the introduction of the larvae, 10 trays of each treatment and 10 trays of unpoisoned soil were removed to the laboratory and examined to determine the percent of the larvae poisoned at each time interval.

The mortalities obtained with the 500- and the 1,000-pound treatments during the fall, winter, and spring under variable conditions in the field at Moorestown, N. J., were plotted against the summations of the thermal units. The scatter diagrams of this relationship are shown in figure 6 by open circles. It was evident that these meager data were not sufficient to establish the shape of any curves, but it was found in both cases that two-thirds of the values were within the limits of the standard errors of the constant-temperature curves. This suggests that a curve derived from data obtained in the field would be within the limits of error of a curve derived under controlled conditions. Since there was no evidence of a difference between these curves derived under constant and variable temperatures, it may be assumed properly that the curves derived under controlled conditions would be an adequate expression of the relationship with variable temperatures in the field.

From a study of the data it may be assumed that the level of mortality obtained with a treatment was dependent upon the number of day-degree units accumulated, whether under controlled conditions in the laboratory or under variable conditions in the field.

JOINT FUNCTIONAL RELATION OF POISONING OF LARVAE TO CONCENTRATION OF LEAD ARSENATE AND ACCUMULATED THERMAL UNITS

The poisoning of larvae in the soil is a joint function of the amount of active lead arsenate in the soil and the accumulated thermal units. It was desired to determine the extent to which the degree of poisoning varied with the joint effect of these factors.

As data were available with the newly hatched larvae on only the 500- and the 1,000-pound treatments, it was not possible to establish this relation for all concentrations of lead arsenate up to 1,000 pounds per acre. The joint functional relation with the 500- and the 1,000-pound treatments was determined according to the procedure outlined by Ezekiel (1). The average percentages of the newly hatched larvae poisoned by these treatments with accumulated day-degree units ranging from 50 to 600 are presented graphically in figure 7.

With the third instars data based on over 20,000 individuals were available on lead arsenate treatments ranging by hundreds from 100 to 1,000 pounds per acre. The joint functional relation was determined with these treatments, and the average mortalities obtained with accumulated thermal units ranging from 100 to 1,600 are given in figure 8.

It is evident from figure 7 that a mortality approaching 100 percent of the newly hatched larvae was obtained with the 500-pound treatment and 450 thermal units, and with the 1,000-pound treatment and 300 thermal units. In these experiments more than 600 thermal units seemed to be required before the average first instar in untreated soil was ready to change into a second instar. Ludwig (6, p. 437, 438) found the average first instar stadium was 16.7 days at 25° C. and 29.6 days at 20° C. From these data it was estimated that 618 to 829 thermal units would be required for the completion of the first stadium. It is evident, therefore, that a larva emerging from an egg in soil containing 500 to 1,000 pounds of lead arsenate per acre should die before its first molt.

With the third instars, as shown in figure 8, high mortality did not occur at any temperature with concentrations of lead arsenate of less than 500 pounds per acre, nor with any of the quantities of lead arsenate used, if the accumulated thermal units were less than 600. A mortality of more than 90 percent was obtained with the 700-pound treatment and 1,600 thermal units, with the 800-pound treatment and 1,400 thermal units, with the 900-pound treatment and 1,200 thermal units, and with the 1,000-pound treatment and 1,100 thermal units. In these experiments some of the third instars ceased feeding and began to pass into the prepupal stage when the accumulated thermal units reached 1,600; many of them were in the prepupal stage when the accumulated thermal units amounted to 2,000.

This suggests approximately 2,000 day-degree units are required for the average third instar to complete that instar and pass into the prepupal stage. Ludwig (6, p. 437, 438) found 101.5 days were required at 25° C. and 105 days at 20° C. to complete the third instar and prepupal stages. From these data it was estimated that 2,940 to 3,755 thermal units would be required.

It would be expected that many of the third instars in treated soil would transform into pupae and emerge as adults when the concentration of lead arsenate in the soil is insufficient to cause a high mortality with 2,000 thermal units, or when the treatment is applied after many of the larvae have passed through a considerable part of their third-instar development.

EXPECTED EFFECTIVENESS OF TREATMENTS IN THE EAST

Most of the treatments with lead arsenate for the control of the Japanese beetle in the soil have been carried on in New Jersey, eastern Pennsylvania, southern New York, and Connecticut--areas in which the climatic conditions are comparable. No experiments have been conducted in northern or southern areas where the climatic conditions are different from those of this region, but wherever the condition of the soil is favorable a prediction may be made of the possible effectiveness of a treatment from the temperature records of the locality and the data obtained in this investigation. These estimates for points remote from those where the studies were made are empirical at present because of the meagerness of the biological and insecticidal data.

Within the area longest infested the first instar is the dominant larval form in midsummer; the second instar is dominant in late summer, and the third instars are the most abundant in the fall, winter, and spring. The third instars complete their growth and pass into the prepupal and pupal stages in the late spring and summer. In the extreme southern areas where it is possible that there may be two generations annually and in the extreme northern areas where it is possible that 2 years may be necessary for the insect to complete its cycle, all instars may occur in the soil during most of the year, with no one dominant at any period. It is not possible to consider this complexity of the larval population in this study. It was assumed in making the estimates of the effectiveness of the treatments that the larval population was not so complex and variable but that in the central and northern areas first and second instars occurred during the summer and third instars during the fall, winter, and spring. In the extreme southern area it was assumed that first and second instars occurred in the summer and winter and third instars in the spring and fall.

The initial dosage of lead arsenate is most effective when applied so that the chemical is in the soil at the time the eggs are hatching, because newly hatched larvae are the most susceptible to poisoning, and the temperature of the soil during the summer is the most conducive to successful treatment. When applied in the fall

or spring, the effectiveness of the treatment is reduced by the lower temperature and the greater maturity of the larvae. When the temperature is conducive to successful treatment, there is a good possibility of killing a larva that ingests the poison shortly after completing its second molt, but there is practically no possibility of killing a fully grown third instar that is about to cease feeding and pass into the prepupal stage. It was assumed, for the purpose of this study, that all the larvae completed their second molt on October 1, so approximately 2,000 thermal units would be needed from this date before they would pass into the prepupal stage. In the central and northern areas these larvae would be partly grown by March 1 and reach maturity sometime after the middle of May, but in the extreme southern area it would be expected that these larvae would reach maturity during the late fall or early winter. In the latter area where two broods a year may be anticipated, it was assumed that the second brood would complete its second molt on March 1.

Data on the temperature of the soil are very limited, but there is an abundance of information on the temperature of the air at various localities throughout the Eastern States. At Moorestown, N. J., it has been found that when the ground is not covered with snow there is little difference between the average temperature of the air and that of the soil at a depth of 1 inch. In view of the very high correlation between these temperatures, it appeared that a close approximation of the summation of the soil temperatures for the various localities could be obtained by a summation of the air temperatures.

Based on the data given in the Yearbook of Agriculture for 1941 (7), the eastern part of the United States was divided into zones of equal temperature for the warmest and the coldest periods of the year, namely, July and January. These zones are shown in figures 9 and 10. For the warm period, summations were made of the thermal units at several localities in each zone for the periods between August 16 and October 1, September 1 and October 1, and September 16 and October 1. These summations were corrected for the deviation of the average temperature of the localities in July from the mean temperature of the zone for that month. For the cold period, summations were made in the same manner for each zone during the period from October 1 to May 20 and from March 1 to May 20.

It was assumed that treatments of 500 and 1,000 pounds of lead arsenate per acre had been applied by July 1 at several localities in each of the five summer-temperature zones to destroy the newly hatched larvae. From the summations of the thermal units in these zones, estimates were made of the expected mortality of larvae on October 1 from batches of eggs hatched on August 16, September 1, and September 16. These expected mortalities are given in figure 11.

It was assumed, further, that the control agents in some localities were unable to apply the treatment on July 1, but some of them applied treatments on October 1 and others on March 1, with dosages ranging from 200 to 1,000 pounds per acre. From the summations of the thermal units

in the nine winter zones, estimates were made of the expected mortality of the third instars by May 20 from these treatments. The expected mortalities are given in figure 12.

In those localities where treatments were applied before the eggs hatched, it would be expected that all larvae hatching on or before August 16 throughout the eastern part of the United States would be killed by applications of 500 pounds of lead arsenate per acre. Of the batch hatching on September 1 it would be expected that all the larvae would be poisoned by the 500-pound treatment in summer zones 3, 4, and 5 and with the 1,000-pound treatments also in summer zones 1 and 2. All the larvae that hatched on September 16 in zone 5 would be expected to be killed by the 500-pound treatment and by the 1,000-pound treatment in zones 4 and 5. The mortality caused by the latter treatment would be expected to approach 100 percent in summer zones 2 and 3 by October 1, but complete mortality would not be expected by this date. In zone 1 a mortality of 68 percent would be expected with the 500-pound treatment and 83 percent with the 1,000-pound treatment. However, the probabilities are that those newly hatched larvae still alive in treated soil on October 1 would ingest a lethal dose of the poison before completing their growth.

In those localities where treatment was delayed until October 1, it would be expected that practically complete elimination of the third instars would be obtained with the 500-pound treatment in winter zones 5, 6, 7, 8, and 9, with the 800-pound treatment in zone 4, and with the 1,000-pound treatment also in zone 3. It would be expected that the 1,000-pound treatment would poison 84 percent of the third instars in the soil in zone 2 and 58 percent of them in zone 1.

In those localities where treatment was delayed until March 1, practically complete elimination of the third instars would be expected with the 500-pound treatment in winter zone 9, with the 800-pound treatment in zones 7, 8, and 9, and with the 1,000-pound treatment also in zone 6. With the 1,000-pound treatment a reduction of at least 75 percent could be expected in zone 4, a 50 percent reduction in zone 3, and something less than 50 percent in zone 2.

Thus from a consideration of the temperatures in the eastern part of the country, between the Great Lakes and the Gulf of Mexico and from the Atlantic coast to the Plains, it is anticipated that if the treatment is adjusted to compensate for the differences in soils and if the application is timed so that the poison is distributed in the soil at the time the eggs are hatching, practically complete elimination of the larval population can be obtained with the 500-pound treatment of lead arsenate. When the treatment is not timed for the newly hatched larvae but applied when the larvae have developed to the last instar, variable results must be expected on the current population throughout this region.

SUMMARY

Since 1941 an investigation has been conducted to establish the influence of the temperature of the soil on the effectiveness of lead arsenate against the larvae of the Japanese beetle. In the maze of interacting factors influencing the insecticidal effect, the temperature was found to be one of the most important.

The velocity of poisoning of the first and third instars increased progressively with the increment in the temperature. At 60° F. the velocity was double that at 50°, it was tripled at 70°, and quadrupled at 80°. The rate of poisoning of the newly hatched larva was about four times that of the fully grown third instar.

A temperature of 40° F. appeared to be the empirical threshold above which poisoning of the larvae by lead arsenate became perceptible.

The poisoning of the larvae was correlated with the summation of the day-degree thermal units above this threshold. This relationship prevailed with constant temperatures in the laboratory and to a considerable degree with variable temperatures in the field.

The joint functional relation of the poisoning of the larvae to the concentration of lead arsenate in the soil and the accumulated thermal units was determined. Newly hatched larvae in soil containing 500 pounds of lead arsenate per acre would be expected to be poisoned before the first molt. Third instars can be expected to cease feeding and pass into the prepupal stage when the accumulated thermal units from the last molt reach 2,000. The effectiveness of the poison against this instar is dependent upon the amount of lead arsenate applied and the number of thermal units accumulated after the distribution of the chemical in the soil.

Consideration was given to the temperatures in the eastern part of the United States, between the Great Lakes and the Gulf of Mexico and from the Atlantic coast to the Plains. It is anticipated that when the treatment is adjusted to compensate for the differences in soil, and the application is timed so that the poison is distributed in the soil at the time the eggs are hatching, practically complete elimination of the larval population can be obtained with 500 pounds of lead arsenate per acre. When the treatment is not timed for the newly hatched larvae but applied when the larvae have developed to the last instar, variable results can be expected on the current population throughout this region.

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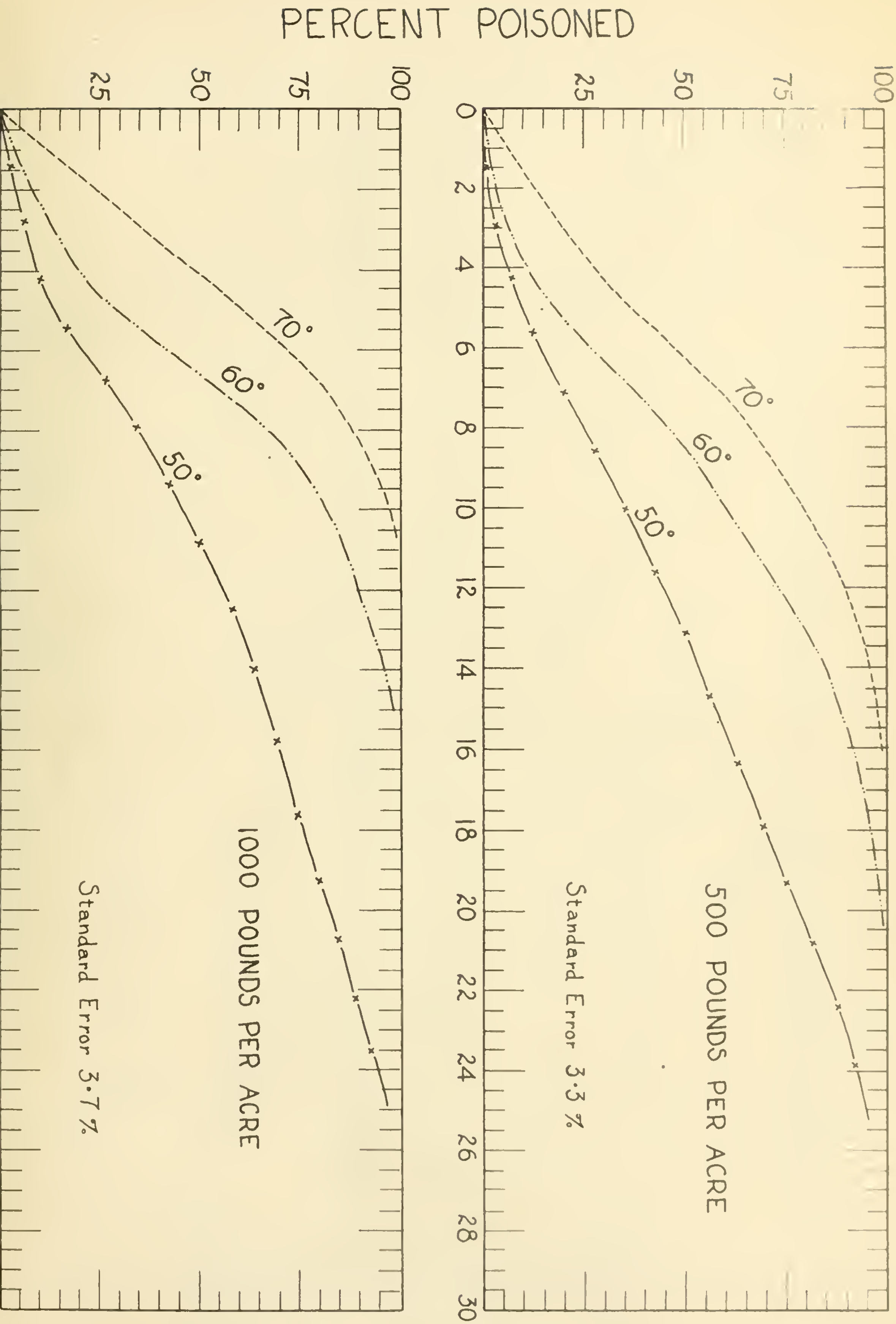

ure 1.— Effectiveness of the 500- and the 1,000-pound treatments
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eetle at constant temperatures of 50°, 60°, and 70° F.

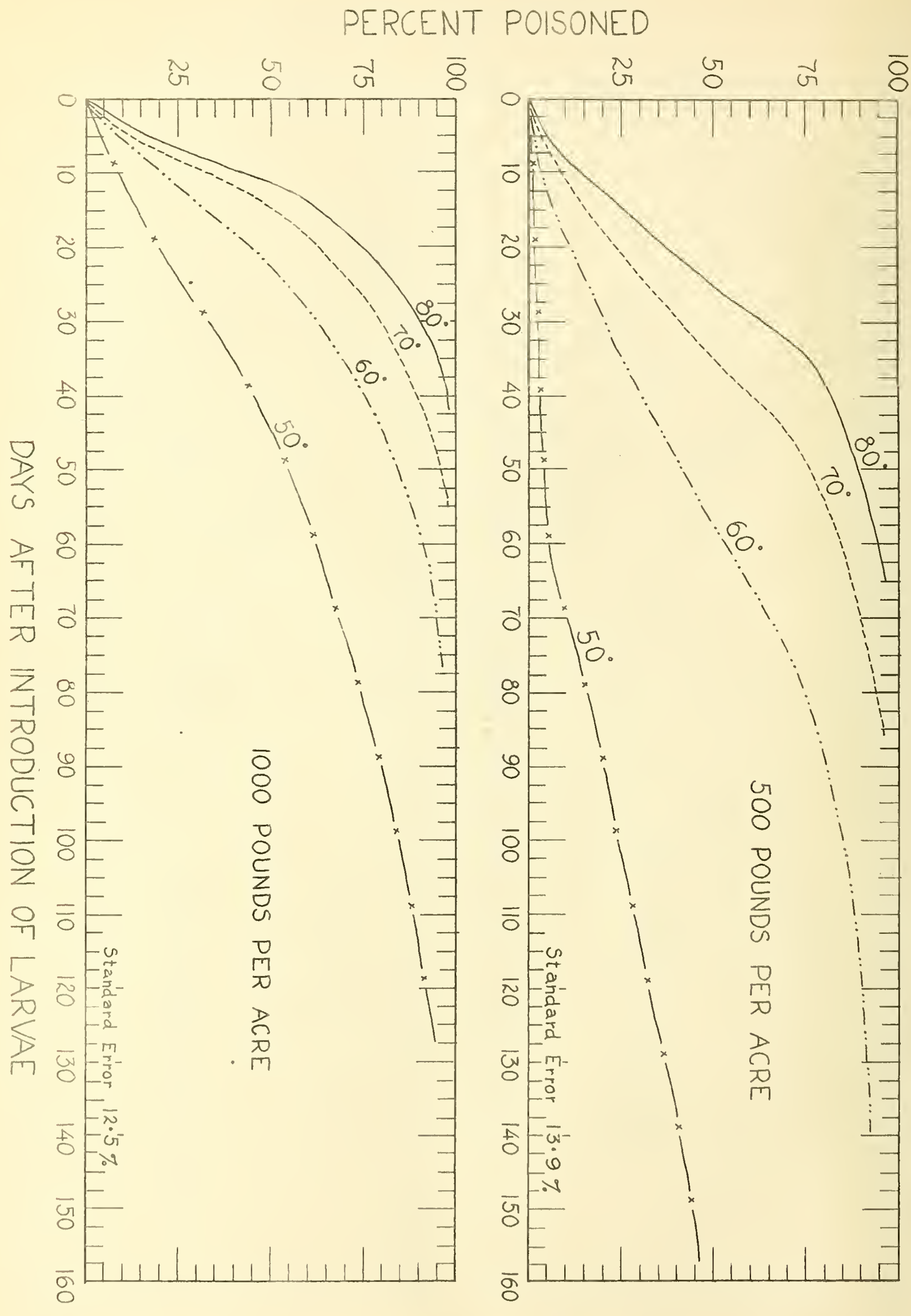

Figure 2.— Effectiveness of the 500- and the 1,000-pound treatments with lead arsenate against third instars of the Japanese beetle at constant temperatures of 50°, 60°, 70°, and 80° F.

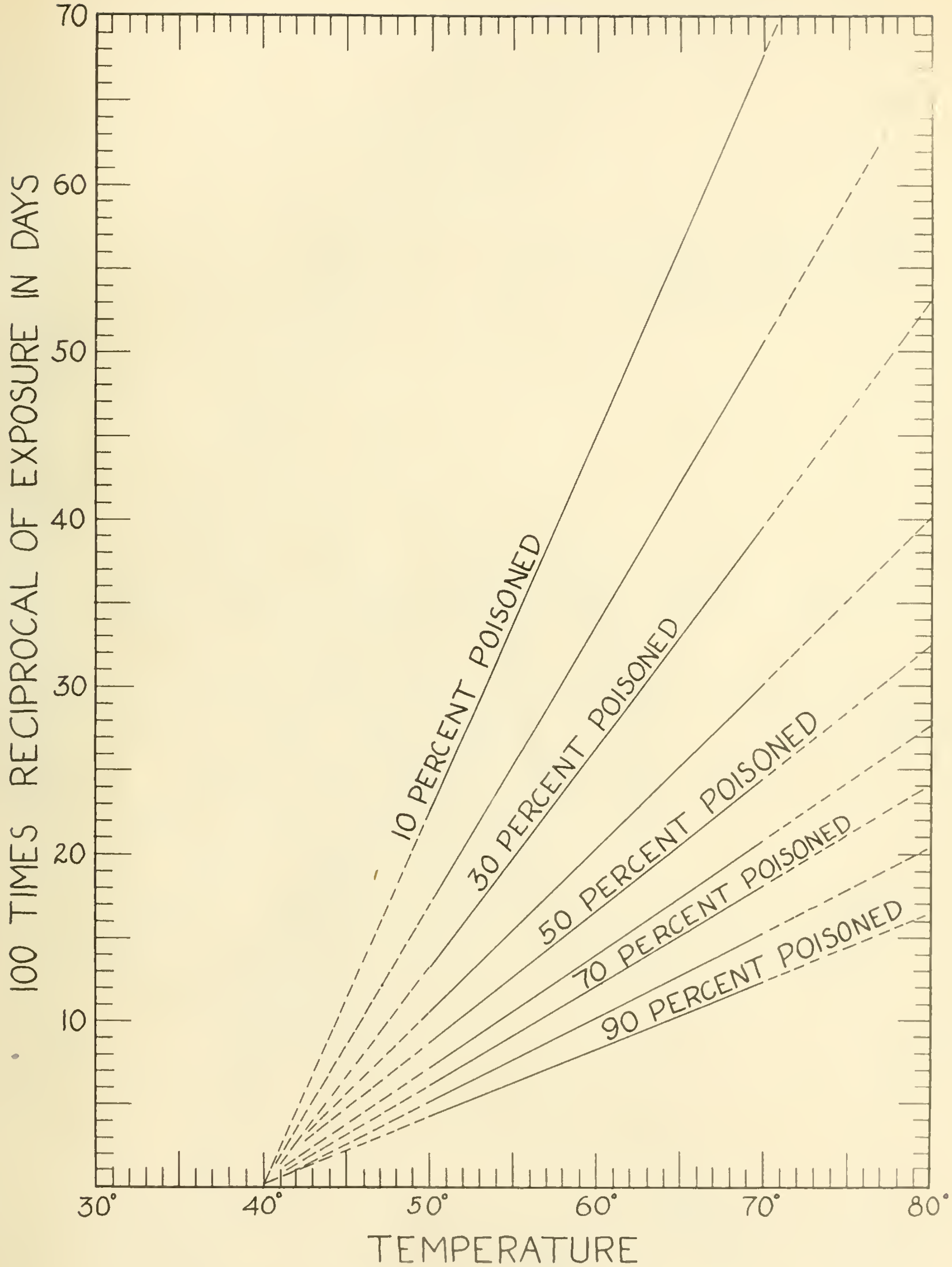

Figure 3.— Relative velocity of poisoning of newly hatched larvae of the Japanese beetle with the 1,000-pounds-per-acre treatment with lead arsenate at temperatures of 50°, 60°, and 70° F., and the empirical threshold temperature of poisoning. (Somewhat diagrammatic.)

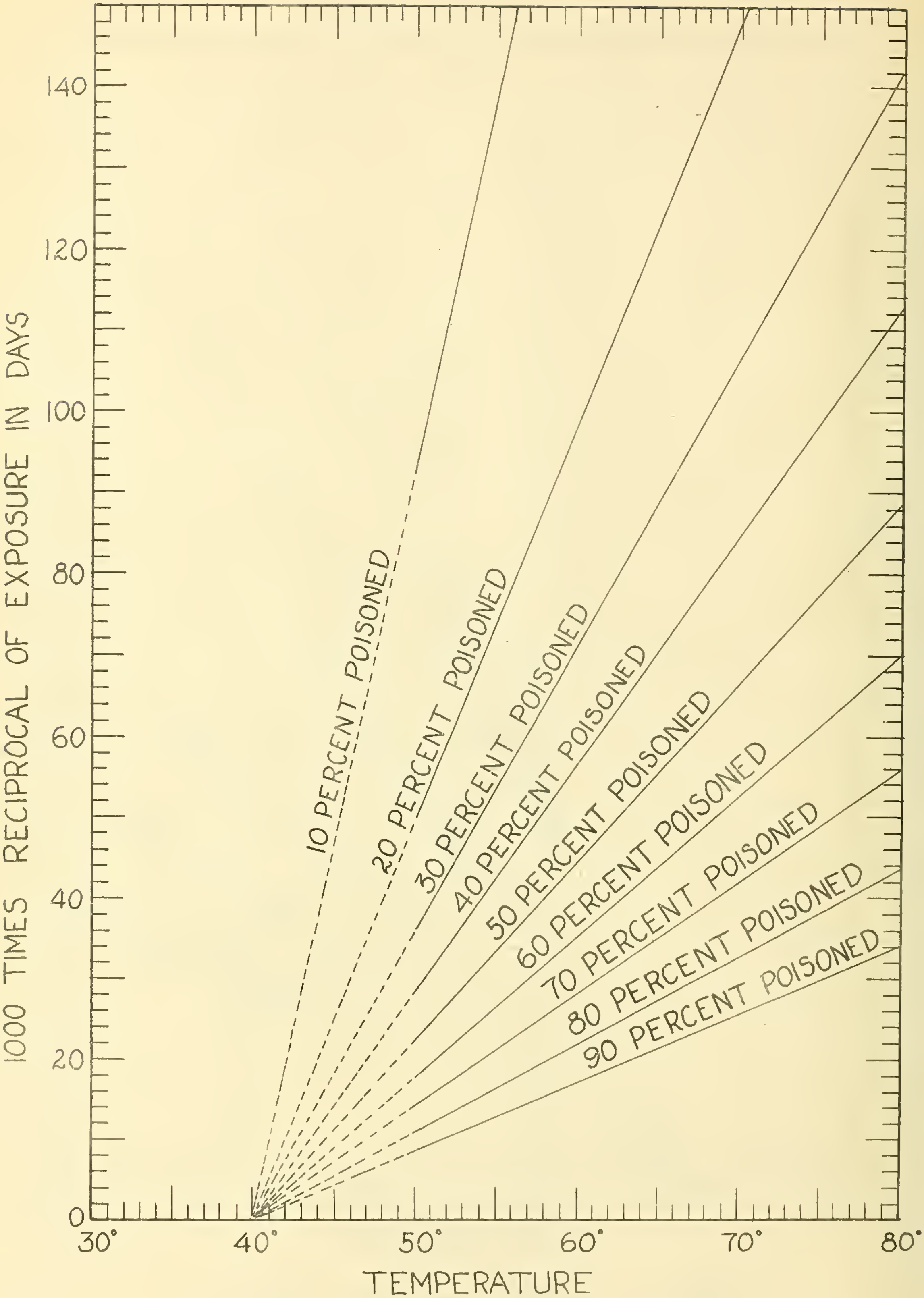

Figure 4.— Relative velocity of poisoning of third instars of the Japanese beetle with the 1,000-pounds-per-acre treatment with lead arsenate at temperatures of 50°, 60°, 70°, and 80° F., and the empirical threshold of poisoning. (Somewhat diagrammatic.)

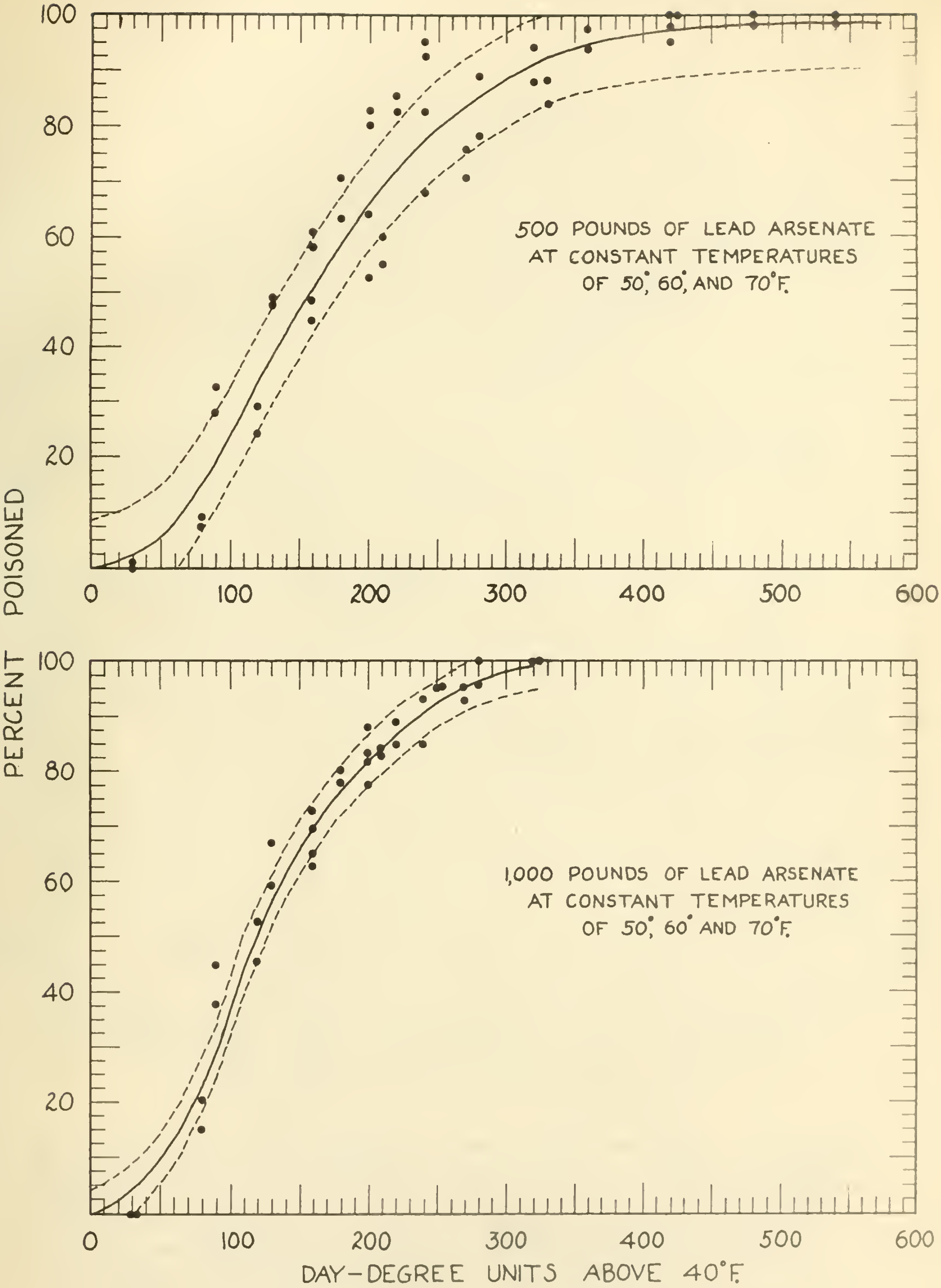

Figure 5.— Relation between the poisoning of the newly hatched larvae of the Japanese beetle with the 500- and the 1,000-pound-per-acre treatments with lead arsenate at constant temperatures and accumulated thermal units above the empirical threshold.

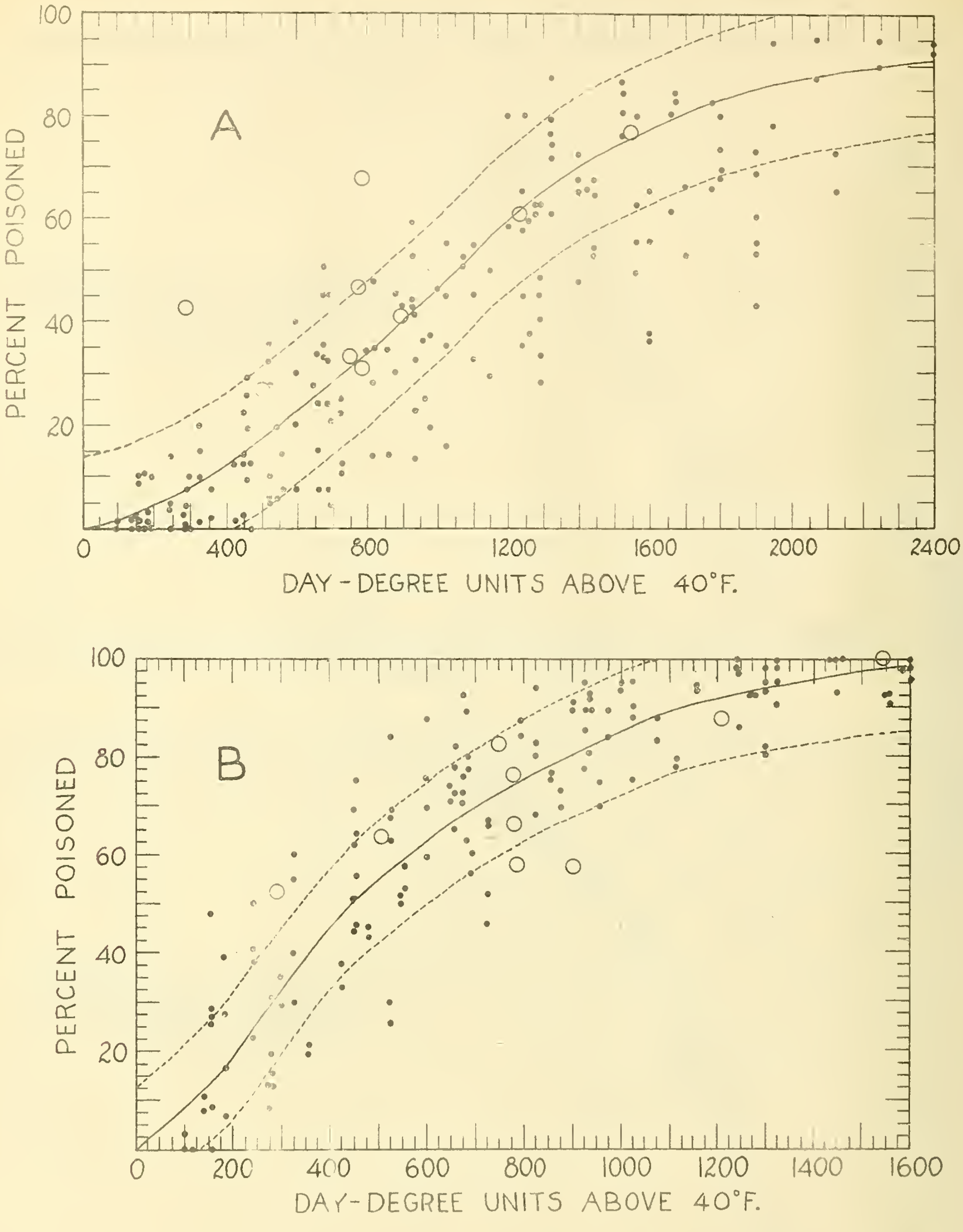

Figure 6.— Relation between the poisoning of third instars of the Japanese beetle with the lead arsenate treatment and accumulated thermal units above the empirical threshold of 40° F. The black dots are the data from constant-temperature experiments carried on at 50°, 60°, 70°, and 80° F. in the laboratory, the solid line is the curve determined by the data, and the broken lines the limits of experimental error. The open circles represent the data obtained from summations of variable temperatures in the field at Moorestown, N.J. Two-third of these circles lie within the limits of experimental error as determined for the constant-temperature data. A, Data for the 500-pound-per-acre treatment; B, for the 1,000-pound-per-acre treatment.

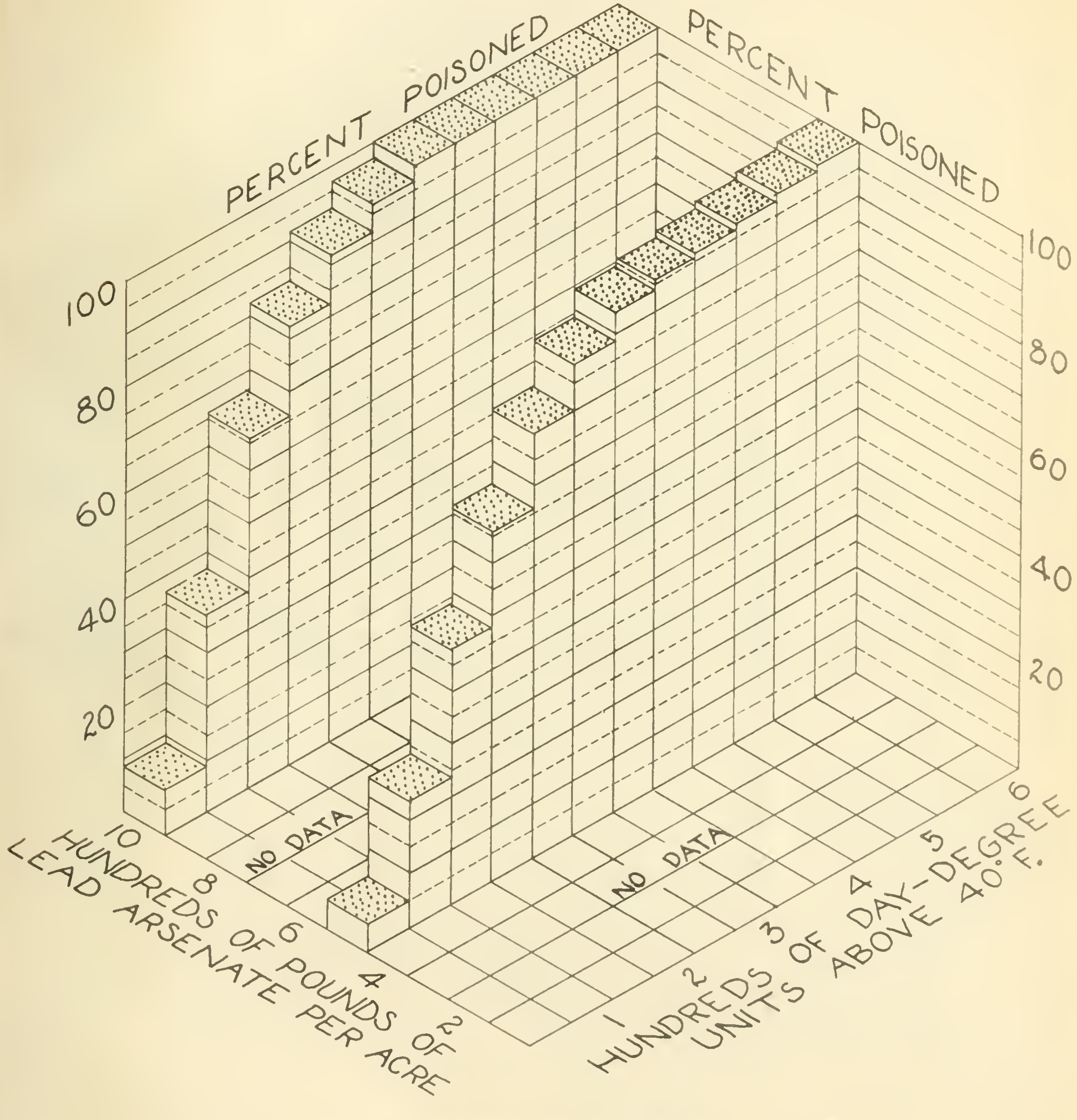

Figure 7.— The joint functional relation between the percentage of the newly hatched larvae of the Japanese beetle poisoned, the quantity of lead arsenate in the soil, and the accumulated thermal units above the empirical threshold.

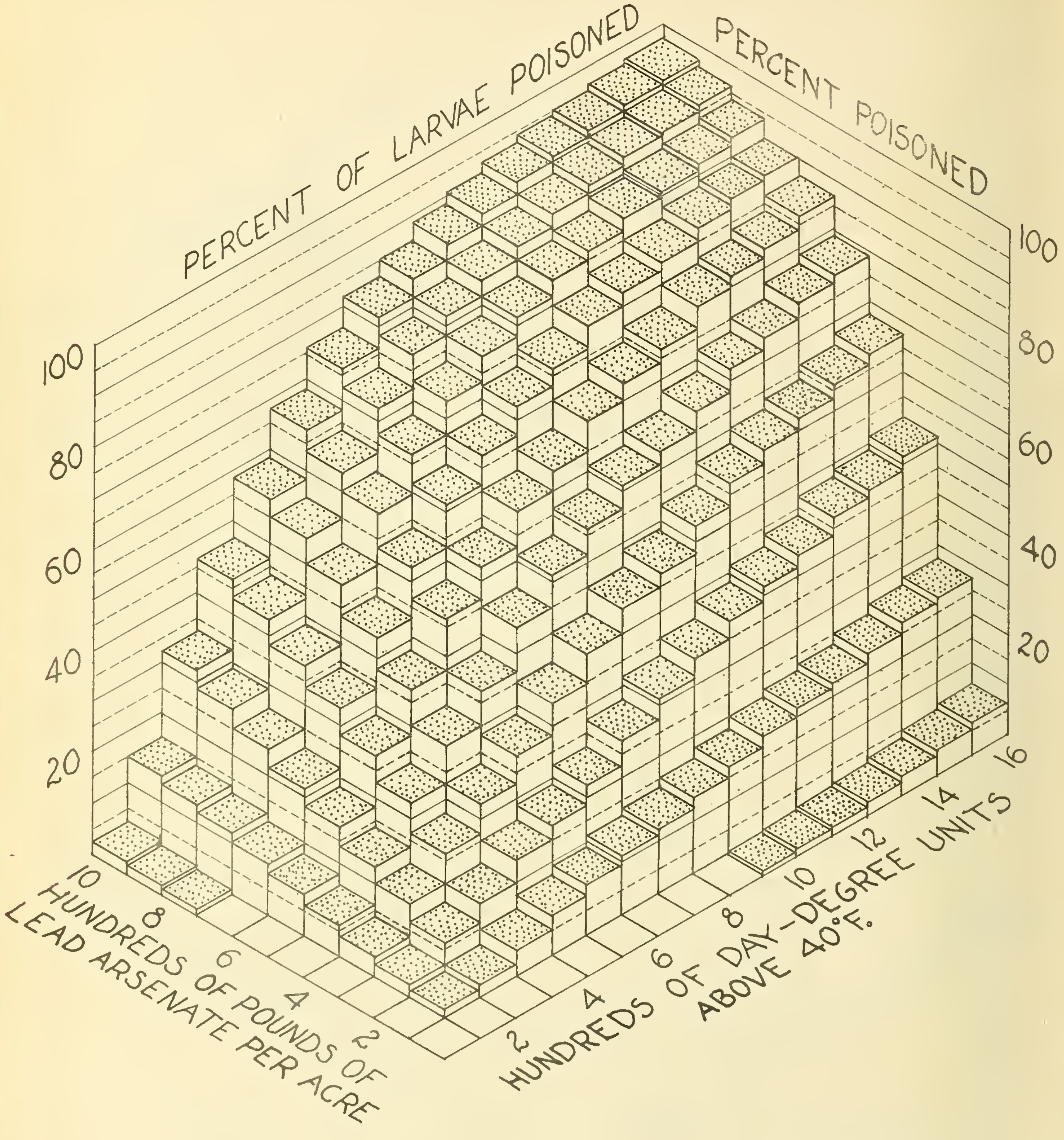

Figure 8.-- The joint functional relation between the percentage of third instars of the Japanese beetle poisoned, the quantity of lead arsenate in the soil, and the accumulated thermal units above the empirical threshold.

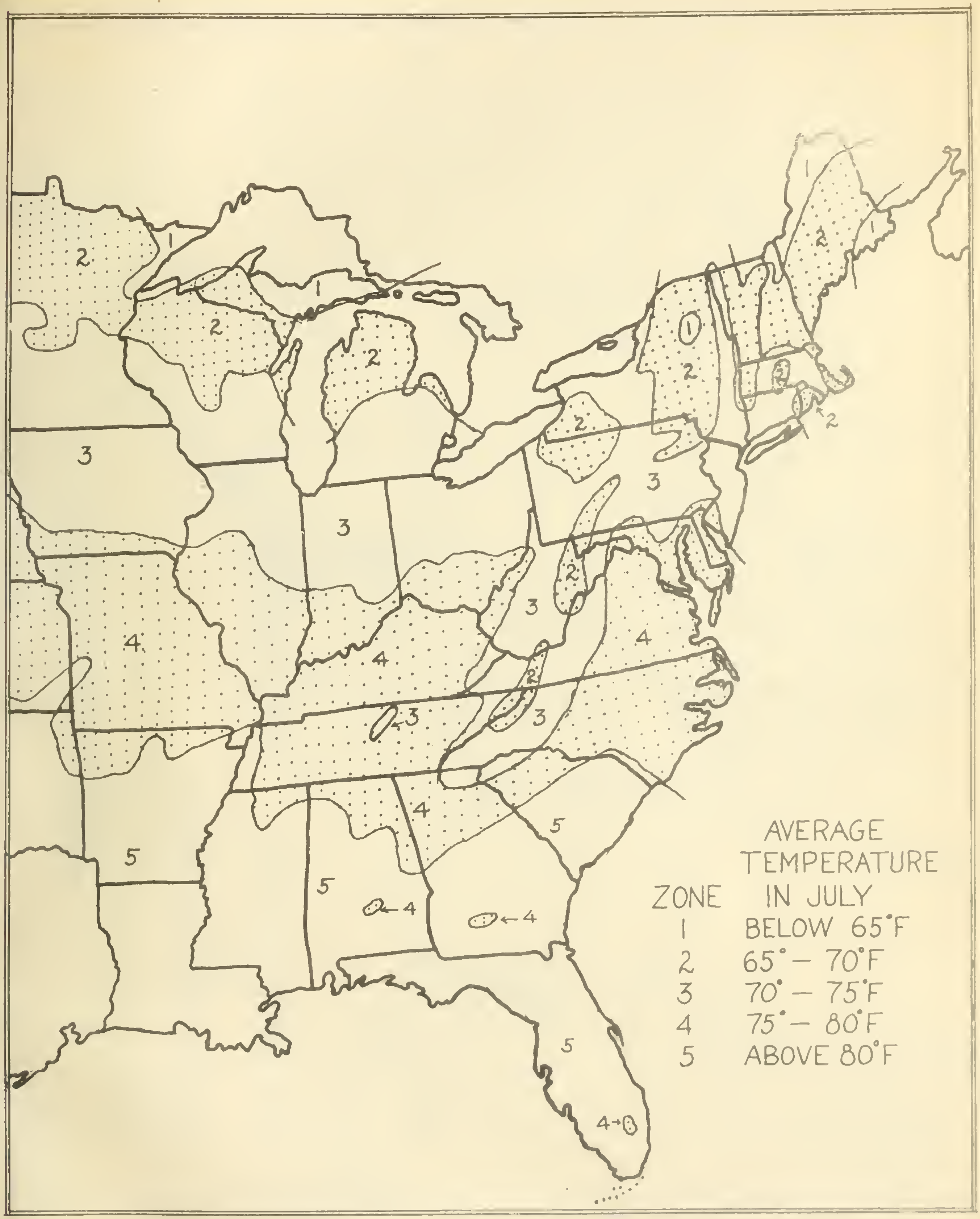

Figure 9.-- Zones of equal temperatures in the eastern part of the United States during the warm months of the year.

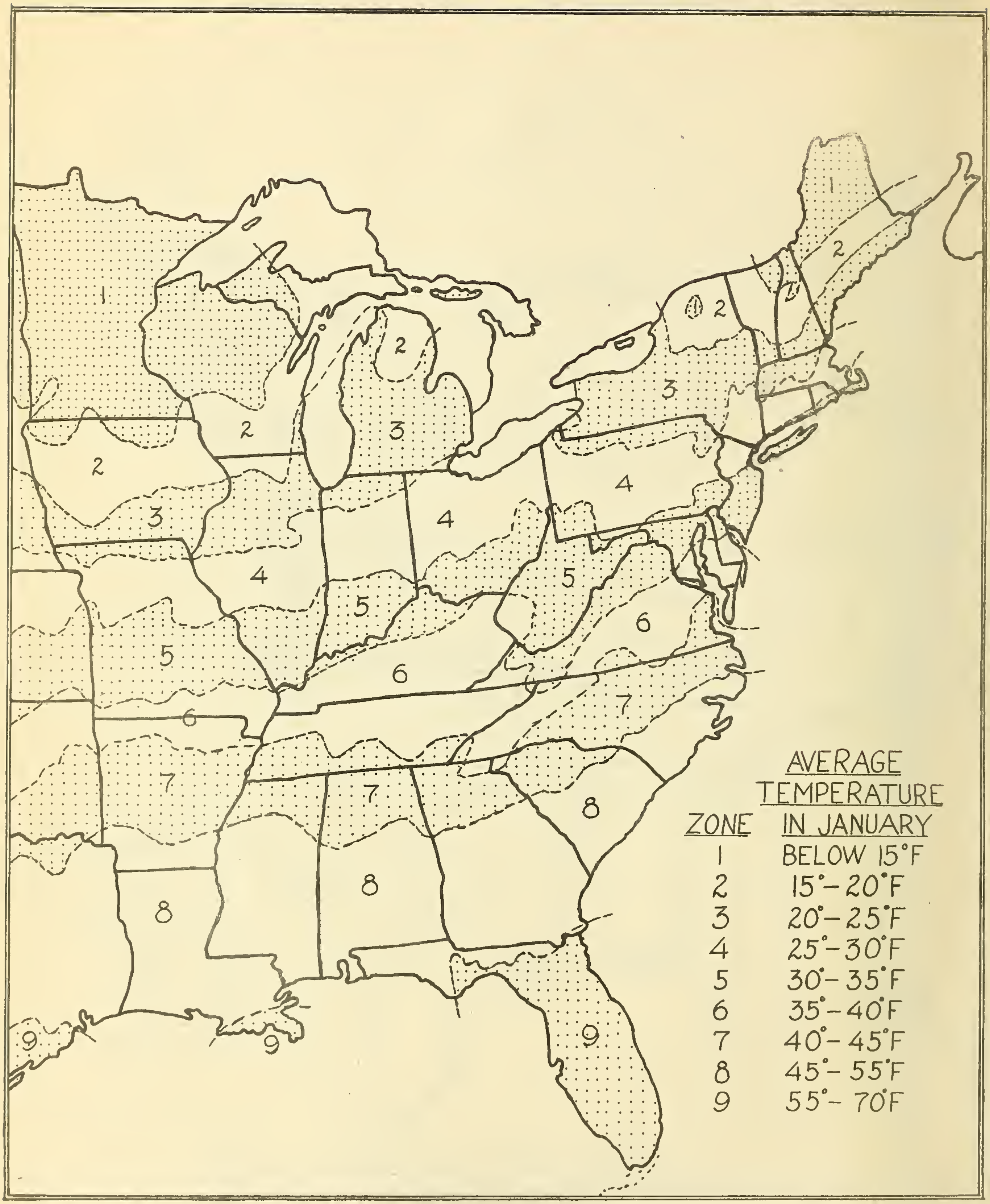

Figure 10.—Zones of equal temperatures in the eastern part of the United States during the cold months of the year.

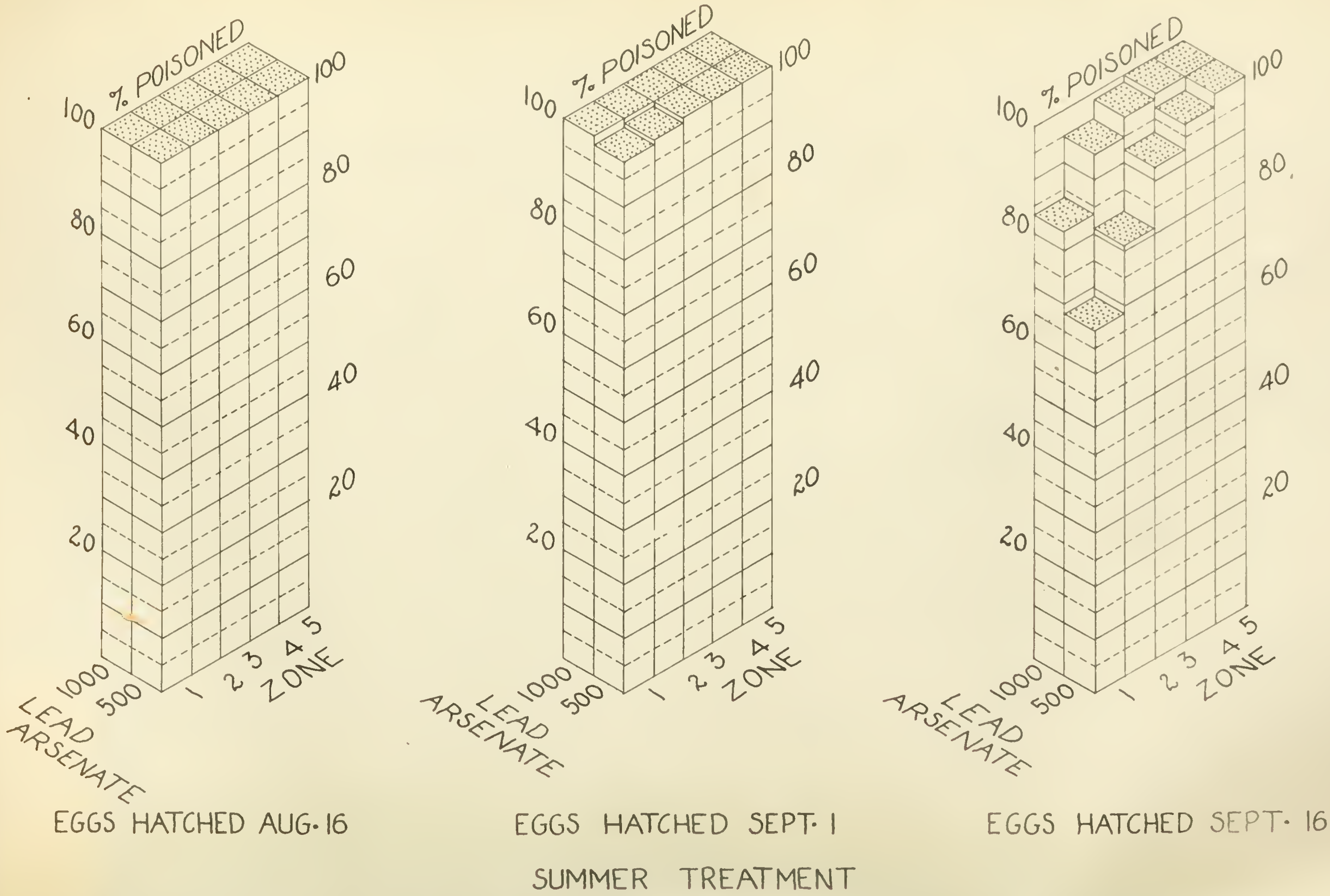

Figure 11.— Expected effectiveness by October 1 of lead arsenate treatments against the newly hatched larvae of the Japanese beetle in the different summer zones of the eastern part of the United States when applied before the eggs hatch in the

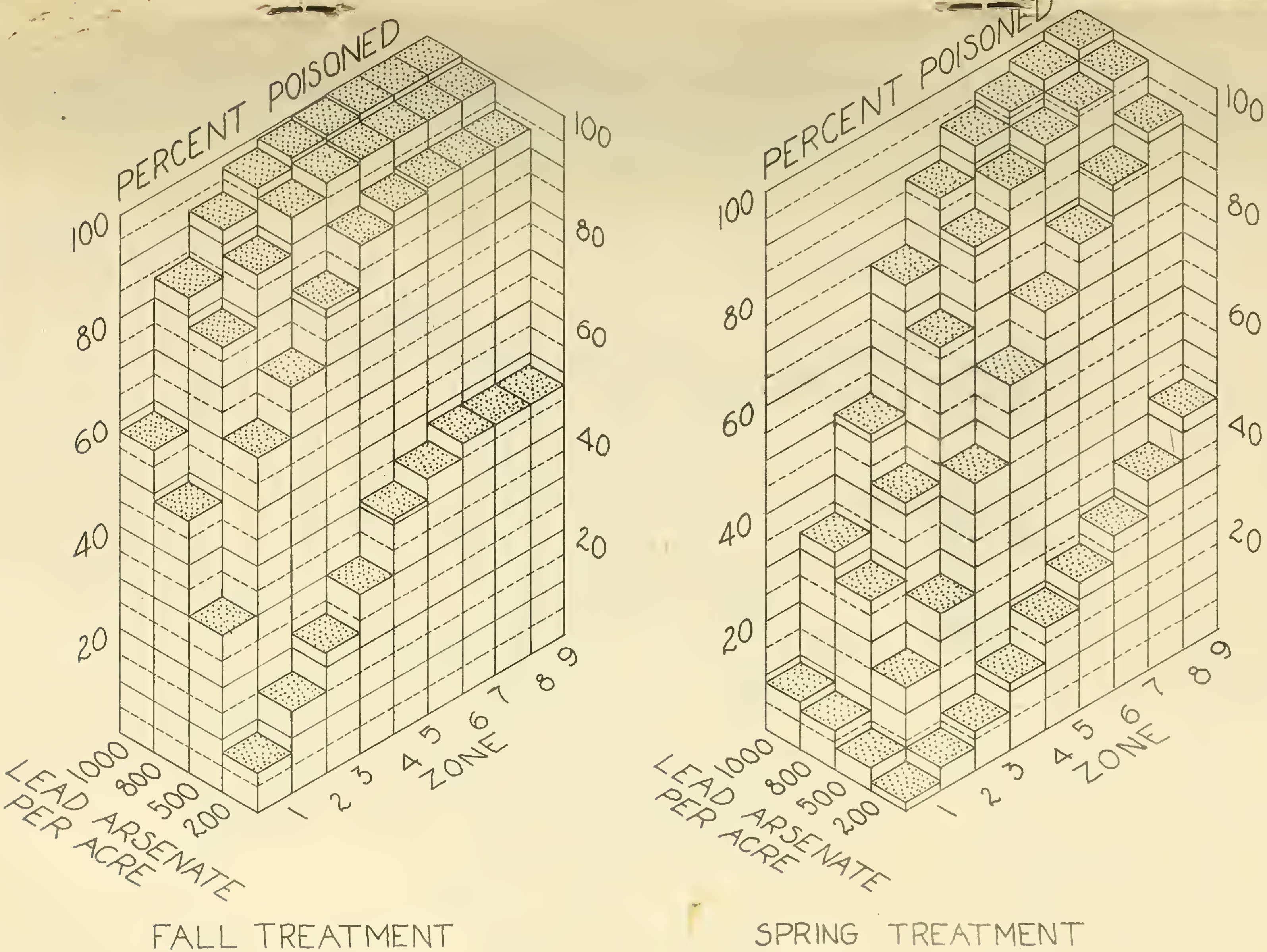

Figure 12.— Expected effectiveness by May 20 of lead arsenate treatments against third instars of the Japanese beetle in the different winter zones of the eastern part of the United States when applied in the fall (October 1) and in the spring (March 1).